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OBSERVATIONS ON THE SPECIFICITY AND THERMOSTABILITY OF THE LIPASE DEVELOPED DURING THE GROWTH OF A RAPIDLY GROWING TUBERCLE BACILLUS IN MEDIA OF VARIED COMPOSITION

STUDIES IN ACID-FAST BACTERIA. VIII *

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The constant presence of a thermostabile lipase, both in the filtrates of broth cultures of certain acid-fast bacilli (including human, bovine, and avian tubercle bacilli, and others) and in the organisms themselves,[1] focuses attention on the possible identity of the lipase produced by these various bacteria. Before this question can be answered, it is desirable to study the effect of the composition of the medium on the nature and extent of the lipolytic activity manifested by a specific organism; for it is conceivable that this lipase may be present in all media in which the given organism will grow, irrespective of composition, or, on the other hand, it may be developed only in the presence of certain substances contained in these media, presumably those on which it can act.

In the first instance, the lipase would be an integral factor in the life history of the organism, whereas in the second case the production of a lipase might be regarded as a definite response on the part of the bacteria to the presence of a specific substance, or substances, in the substrata on which the organisms have grown. If the first assumption is correct, lipolytic activity should be demonstrable even in the simplest media compatible with growth of the organisms; such ferment activity, however, would not necessarily rule out the latter possibility.

The experiments here recorded were designed to show the effect of the composition of the medium on the nature and extent of lipolytic activity exhibited by a rapidly growing human tubercle bacillus (597). The media selected for this purpose varied in composition from one of extreme simplicity, containing ammonium chlorid as a source of nitrogen, and ethyl alcohol as a source of carbon, through others of various

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1. See preceding articles.

degrees of complexity to the regulation nutrient broth commonly used for the culture of bacteria, nutrient meat-juice-peptone broth. The qualitative composition of these media is clearly indicated in Table 1, where the sources of nitrogen and carbon are specifically set forth. The composition of the nutrient broths, except for the addition of various carbohydrates, is omitted for obvious reasons. The organism was grown for several successive transfers in each of these media before the lipolytic activity was tested, both to acclimatize it to the various ingredients and to insure maximum growth. The final determinations recorded below were made uniformly after twenty-one days' incubation at 37 C. The technic has been described previously.

Throughout this work controls have been made in the following manner:

Media Controls.—One cubic centimeter of the sterile uninoculated medium was mixed with 10 c.c. of sterile distilled water, and, after the introduction of the ester and toluene, incubated under parallel conditions with the corresponding lipase determinations in the same medium.

Ester Controls.—The appropriate amounts of ester and toluene suspended in 10 c.c. of water were likewise inoculated at 37 C. with the media controls and the lipase solutions. In order to save unnecessary complications in the table, it can be stated definitely that, without exception, both the media controls and the ester controls showed no change in reaction after incubating twenty-four hours in the manner indicated. That is to say, whatever changes are noticed in the lipase-containing solutions are due to the action of the ferment and not to any reactionary changes attributable either to the culture medium itself or in the esters.

Vigorous attention to the cleanliness of all apparatus coming in contact with the culture media has been observed. The organism was grown for several successive transfers in the medium in which the observations were subsequently made. This is a point of considerable importance, for the procedure eliminates the possibility of transfer of active substance at the time of inoculation.

With this explanation, the results shown in Table 1 are self-explanatory. The figures in the table represent the cubic centimeters of N/50 NaOH required to neutralize the acid liberated from the various esters after twenty-four hours' incubation with 1 c.c. of the clear medium underlying the pellicle of bacteria, which forms as the organisms grow. The total lipase activity of each culture is, therefore, theoretically, one hundred times the amount indicated in the table, for it will be remembered that but 1 c.c. of the culture medium is used in these determinations, while the total volume of culture in which the organisms have grown is 100 c.c. in each case. Generally speaking, the results show that the extent of the lipolytic activity increases with

TABLE 1

LIPOLYTIC ACTIVITY OF TUBERCLE BACILLUS 597

Days of Growth	Composition of Media			Cubic Centimeter of N/50 NaOH to Neutralize							
	Source of Nitrogen	Source of Carbon	Other Ingredients	Methyl Salicylate	Ethyl Acetate	Ethyl Butyrate	Amyl Acetate	Amyl Butyrate	Amyl Valerianate	Triacetin	Castor Oil
21	NH_4Cl	CH_3CH_2OH	Na_2HPO_4NaCl	....	....	0.25	....	....	....	....	....
21	NH_4Cl	Glycerin	Na_2HPO_4NaCl	....	0.20	0.40	0.70	0.40	0.50	....	....
21	NH_4Cl	Dextrose	Na_2HPO_4NaCl	....	0.20	0.35	0.40	0.65	0.45	....	....
21	NH_4Cl	Mannite	Na_2HPO_4NaCl	....	0.30	0.40	0.40	0.65	0.55	....	....
21	Glucose	Amine	Na_2HPO_4NaCl	....	....	0.25	....	....	....	....	....
21	$(NH_4)_2HPO_4$	CH_3CH_2OH	NaCl	....	....	0.25	....	....	....	....	....
21	$(NH_4)_2HPO_4$	CH_3COONa	NaCl	....	0.55	0.55	0.60	0.45	0.50	....	....
21	$(NH_4)_2HPO_4$	Glycerin	NaCl	0.20	0.40	0.30	0.40	0.20	0.35	....	0.35
21	$(NH_4)_2HPO_4$	Dextrose	NaCl	0.20	0.25	0.40	0.45	0.20	0.40	....	0.40
21	$(NH_4)_2HPO_4$	Mannite	NaCl	0.25	0.30	0.55	0.25	0.50	0.50	....	0.50
21	Asparagin	CH_3CH_2OH	Na_2HPO_4NaCl	....	....	0.30	....	....	....	....	....
21	Asparagin	Glycerin	Na_2HPO_4NaCl	0.40	0.85	0.60	0.70	0.40	0.30	0.40	0.90
21	Asparagin	Dextrose	Na_2HPO_4NaCl	0.50	0.70	0.70	0.80	0.45	0.40	0.50	0.40
21	Asparagin	Mannite	Na_2HPO_4NaCl	0.50	0.70	1.10	0.95	0.50	0.25	0.50	0.50
21	Asparagin $(NH_4)_2HPO_4$	CH_3CH_2OH	Na_2HPO_4NaCl	....	....	0.30	....	....	....	....	....
21	Asparagin $(NH_4)_2HPO_4$	Glycerin	Na_2HPO_4NaCl	0.60	0.80	0.55	0.80	0.50	0.70	....	....
21	Asparagin $(NH_4)_2HPO_4$	Dextrose	Na_2HPO_4NaCl	0.30	0.65	0.45	1.00	0.65	0.85	....	....
21	Asparagin $(NH_4)_2HPO_4$	Mannite	Na_2HPO_4NaCl	0.50	0.80	0.60	0.85	0.45	0.75	....	....
21	Nutrient broth	Plain	.....	....	....	0.85	....	....	....	....	....
21	Nutrient broth	Glycerin	.....	....	....	1.95	....	....	....	....	....
21	Nutrient broth	Dextrose	.....	....	....	1.55	....	....	....	....	....
21	Nutrient broth	Mannite	.....	....	....	1.75	....	....	....	....	....
	Control	.....	.....	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

increased complexity in composition of the medium in which the organism is grown. The lipolytic activity, observed in media in which ammonia, either as ammonium chlorid or diammonium hydrogen phosphate, is a source of nitrogen, is distinctly less than that observed in nutrient broth where amino-acids, and probably other nitrogen-containing bodies, are possible sources of nitrogen. The source of carbon appears to make but little difference in the extent of lipolytic activity, at least in the simpler media where this relationship can be definitely observed. The relative luxuriance of growth of the tubercle bacillus in the various media presented above leads one to believe that the extent and thickness of the pellicle, that is, the luxuriance of the growth of the organism, varies almost directly with the increasing complexity in

TABLE 2

EFFECT OF HEATING LIPASE TO 100 C. FOR 15 MINUTES

Days of Growth	Source of Nitrogen	Source of Carbon	Other Ingredients	Unheated Filtrate Cubic Centimeters of N/50 NaOH					Heated Filtrate (100 C.-15') Cubic Centimeter of N/50 NaOH				
				Ethyl Acetate	Ethyl Butyrate	Amyl Acetate	Amyl Butyrate	Amyl Valerianate	Ethyl Acetate	Ethyl Butyrate	Amyl Acetate	Amyl Butyrate	Amyl Valerianate
22	$(NH_4)_2HPO_4$	Sod. Lactate	NaCl	0.55	0.60	0.60	0.45	0.50	0.60	0.50	0.35	0.45	0.50
22	$(NH_4)_2HPO_4$	Glycerin	NaCl	0.40	0.50	0.70	0.40	0.50	0.45	0.40	0.80	0.30	0.60
22	$(NH_4)_2HPO_4$	Dextrose	NaCl	0.40	0.35	0.40	0.65	0.45	0.25	0.50	0.70	0.35	0.45
22	$(NH_4)_2HPO_4$	Mannite	NaCl	0.30	0.35	0.40	0.65	0.55	0.25	0.45	0.50	0.45	0.60

composition of the medium. Therefore, the conclusion that the extent of lipolytic activity varies almost directly with the relative luxuriance of growth appears to be justified. On the other hand, the action of the lipase is about the same on the various esters tested, irrespective of the composition of the medium. This observation is in harmony with the extensive observations of Loevenhart,[2] who has shown that the same lipase can act on a variety of esters.

It is apparent from the results that the lipase produced by the tubercle bacillus can act on various esters, even tho it is developed by the organisms grown in media of the simplest composition. It should be again emphasized that the organisms were repeatedly trans-

2. Jour. Biol. Chem., 1907, 2, p. 456.

ferred in these simple media before the results presented here were obtained, so that there is practically no possibility that a transfer of lipase, other than that developed by the organisms in the medium under question, can have taken place at the time of inoculation.

Table 2 shows the effect of heating the lipase solution to 100 C. for fifteen minutes before adding the ester and toluene. The results indicate that the lipase demonstrable in the simplest media is thermostabile, agreeing in this respect with the thermostability of the lipase found in the more complex nutrient broth. This is another argument in favor of the identity of the lipase developed in these various media.

CONCLUSIONS

A rapidly growing strain of the human tubercle bacillus produces a lipase which appears to be qualitatively the same when it is grown in media varying in composition from one consisting essentially of ammonium chlorid, ethyl alcohol, Na_2HPO_4, and NaCl, to the extremely complex nutrient meat-juice-peptone broth ordinarily used for bacterial cultures.

The lipase observed in the simplest media acts on various esters, irrespective of the nature of the carbon compound of the medium in which it is developed. For example, the lipase developed in the $(NH_4)_2HPO_4$ mannite medium acts on triacetin and castor oil as energetically as on ethyl butyrate, or other simple esters. That is to say, the lipase developed in the simplest medium acts even on a complex glycerid.

This lipase appears to be thermostabile whether it is tested in the simplest media or in the most complex.

The activity of the lipase appears to be roughly proportionate to the relative luxuriance of the growth of the tubercle bacillus.